BIO-JET FUEL PRODUCTION FROM THE ENHANCED CROPS OF *BOTRYOCOCCUS BRAUNII* BY MgO NANOPARTICLES (MgO NPs)

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Abstract

One of the most pressing challenges facing humanity at this time, and that will surely define our future in a few years, is that of energy. The finite nature of fossil fuels and concerns regarding environmental impact, especially on greenhouse gas (GHG) emissions, have established the need to search for new energy sources.

Biofuels from biomass will cover a large part of the energy demand required for transport. The economic and environmental impacts of the production of biofuels have recently been studied, highlighting the importance of knowledge biodiesel and bio-jet fuel (bio-kerosene) production because they are technologies capable of replacing the consumption of fuels derived from petroleum, mainly those destined for the transport sector. Bio-jet fuel can be used in aircraft engines without modification.

In this work, bio-jet fuel was obtained from the lipids of the microalgae *Botryococcus braunii*, cultivated in pre-treated wastewater and adding MgO NPs to improve lipid and / or biomass production in the crop. Carrying out this type of research the challenges, and opportunities what this technology represents for its implementation in developing countries is of great environmental relevance, since the combustion of energy is responsible for just over 61% of CO_2 emissions and 46% of GHG, according to whit global stock take.

Keywords: Microalgae, crop improvement, domestic wastewater, metallic nanoparticles, non-fossil fuels.

1. Introduction

After a decade of record passenger traffic growth and unprecedented profitability, the global airline industry is facing an unprecedented, sharp, and sustained drop in demand from the coronavirus pandemic. Since the global lockdown began, the number of daily flights has fallen by between 90 and 95% in some regions and almost all passenger traffic has been suspended worldwide [1].

Under these circumstances, the optimal development of the transportation industry in general, and particularity aeronautics, in addition, should reduce its greenhouse gas emissions (GHG) and its high dependence on fossil fuels; It should be noted that in 2009, the aviation industry put in place an ambitious and robust carbon emissions, the goal aims to achieve carbon neutral growth starting in 2020 and achieve the goal of reducing net carbon emissions by 50% by 2050 [2].

One of the most promising strategies is to develop and industrialize alternative aviation fuels produced from renewable resources, like biomass [2, 3].

Biomass is the organic material that has been most used as fuel throughout the entire history of humanity. It is produced by plants by fixing light, water, and carbon dioxide through the photosynthesis process: Solar energy is stored in chemical bonds, and can be released through processes such as combustion, digestion, decomposition or through its hydrolysis and fermentation to liquid or gaseous fuels. Using biomass as an energy source is one of the most promising ways to reduce energy dependence on non-renewable fossil resources, while reducing the carbon footprint [4,5].

Biofuels are those produced from biomass, and which are considered as a renewable energy source; can be presented in solid (vegetable waste, biodegradable fraction of urban or industrial waste), liquid (bio-alcohols, biodiesel, bio-jet fuel) and / or gaseous (biogas, hydrogen) forms [4].

Biofuels can be classified according to the biomass used for their production in first, second and third generation. In the first-generation food crops were used that endangered food security, in the second-generation non-food crops were used, but they still occupied farmland and in the third-generation microorganisms such as algae, bacteria or yeasts produced at big scale. (Figure 1).

Currently on a commercial level, mass cultivation of microalgae is of greater importance to produce high purity chemical compounds, such as: biofuels, biofertilizers, ion exchangers and carotenes; likewise, for the treatment of

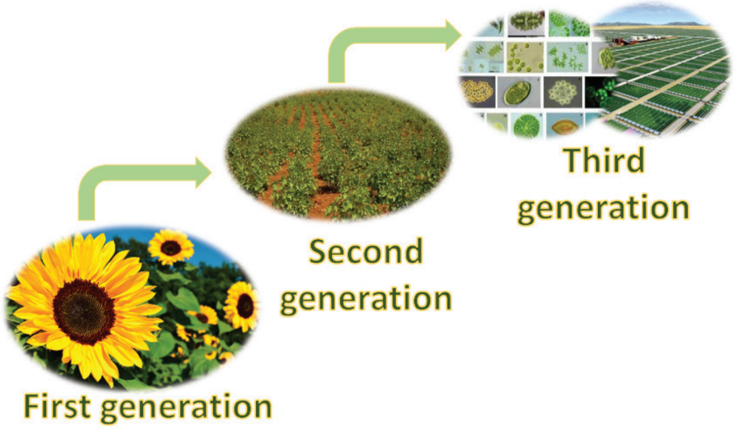

Figure 1. Evolution of the biomass used in the production of biofuels.

wastewater, obtaining therapeutic compounds and as food for human and animal consumption [6].

Despite the existence of technologies to capture solar, hydro and wind energy, the use of liquid and gaseous biofuels from biomass will cover a large part of the energy demand required for transport (Balat 2011). Also, the production of these biofuels offers environmental advantages such as the reduction of emissions (GHG) by up to 70-90% [7].

As far as nanoparticles, strictly speaking, nanoparticles are those particles between 1 to 100 nm, where the physical properties of solid materials change dramatically, they have very different physical and chemical properties from the same materials on a conventional scale. Its properties depend on its shape, size, surface characteristics and internal structure. Another fundamental aspect of nanoparticle synthesis is its stabilization, so that its size and shape can be maintained over time [8 - 11].

Concerning microalgae and biofuels [12], carried out a study on the behavior of *Chlorella pyrenoidosa* and on how to accelerate its metabolism by adding CaO and MgO | to stimulate lipid production, because for plant growth and development, MgO is essential [13]. Nanomaterials are added since the microalgae are cultivated to stress it and have a higher percentage of lipid production than just using sugars from CO_2.

Therefore, in this work, bio-jet fuel was obtained from the lipids of the microalgae *Botryococcus braunii*, cultivated in pre-treated wastewater and adding MgO nanoparticles to improve lipid and / or biomass production in the crop.

2. Methodology

2.1. *Materials and reactives*

All the reagents used were analytical grade; also, all analyzes were carried out in triplicate.

2.2. *Botryococcus braunii*

The choice of species is the first step in the development of a production process. Among the main desirable characteristics for large-scale crops are rapid growth, elevated content of high added value products, development in extreme environments, large cells in colonies or filaments, great tolerance to environmental conditions, tolerance to high levels of CO_2 (15 % or more), to contaminants and to the physical effect of agitation or turbulence [14].

In this work the algae species *Botryococcus braunii* was used, due to its high lipid content (Table 1). These types of algae are useful to produce biofuels, chemicals, or chemical precursors. For commercial production of these compounds, adapted algal strains and optimized growing conditions are mandatory [15].

Species	**Lipid content (% dry biomass weight)**
Botryococcus braunii	25-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Monallanthus salina	20

Table 1. Lipid content of some species of microalgae.
Modified from [15].

This species is notable for its ability to produce large amounts of hydrocarbons, especially oils in the form of triterpenes, hydrocarbons known as *botryococcenes,* which come to represent around 30-40% of its dry weight, that can be used as renewable feedstocks for producing combustion engine fuels [16].

The strain to this work was purchased from the University of Texas in the USA with the code UTEX B 2441 (Figure 2).

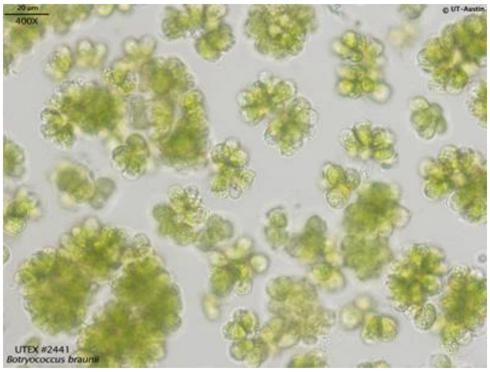

Figure 2. *Botryococcus braunii* UTEX 2441.
(Image obtained from the UTEX culture collection).

The cultivation conditions throughout five months, were:

- Light intensity 33 µE / m2s1
- Stirring 125 rpm
- Photoperiods 16/8
- pH 6.7 - 7.2
- Temperature 25 ± 1 ° C

The optical density (OD) was monitored weekly using the Perkin Elmer Lambda 35 UV-Vis spectrophotometer, at a maximum wavelength of 683.6 nm, to determine the growth kinetics of this microalgae. In this study we determined that optical density, since other authors like kawamura *et al* 2021, reported an optical density of 660 nm for *B. braunii* [17].

Botryococcus braunii UTEX 2441 was cultivated in domestic wastewater, obtained from the pilot plant for wastewater treatment of the UAM-Azcapotzalco and this water was characterized using physicochemical methods and sterilized in a vertical laboratory autoclave of soil model ATV 850; considering that comes from human feces and urine, from personal and kitchen care and house cleaning, they usually contain a large amount of organic matter and microorganisms, as well as traces of soaps, detergents, bleach and fats, among others [5].

2.3. *MgO Nanoparticles (MgO NPs)*

MgO NPs synthesis was performed by the method described by Chavez-Sandoval et al 2021 b [11], with some modifications briefly: A stock solution of 250 mM magnesium oxide was prepared in deionized water; 6.5 mL of this solution was taken to reach a volume of 650 mL with a concentration of 2.5 mM. A reflux system was installed using an Erlenmeyer flask on a heating grill and constant stirring until boiling was reached. 16.25 mL of 1% sodium citrate (Na3C6H5O7·2H2O), was added as a reducing and stabilizing agent. It was kept boiling and stirring for 15 minutes. Afterward, it was transferred to a grill with only stirring to cool for at least 15 minutes. Finally, it was stored at 4°C until use.

The MgO NPs were characterized by UV-Vis Spectrometry techniques, using a Perkin Elmer UV 35 spectrophotometer, by scanning from 200 to 235 nm.

MgO NPs were added to the 1000 mL photobioreactors in concentrations of 5 %V, 10 %V and 20 %V. A photobioreactor was used as a blank, no MgO NPs were added (Table 2).

Photobioreactor	Concentration of MgO NPs %V
1	0 (Blank)
2	5
3	10
4	20

Table 2. Addition of OMg NPs in the photobioreactors

2.4. *Microalgae harvest*

Centrifugation, sedimentation, filtration, and flocculation, either individually or in combination, are the most practical harvesting procedures, whose application depends on the properties of the cultivated microalgae species (morphologies, presence of gaseous vacuoles, etc.), since some have characteristics that facilitate its collection [18]. Oils are extracted from the harvested biomass, using organic solvents, mainly hexane.

Biomass was obtained by centrifugation in this work, an Eppendorf brand Centrifuge 5810R centrifuge was used, at 4000 rpm for 4 minutes and at a temperature of 4 °C. The technique used to obtain the biomass can also be first by filtration and then by centrifugation, depending on the volume of the sample.

2.5. *Microalgae drying*

The biomass obtained was placed in Petri dishes previously sterilized and weighed for drying. Drying was carried out using a Binder brand redLine culture oven at 29 °C for 12 hours.

2.6. *Transesterification*

The transesterification reaction is the most usual form of biodiesel production. This reaction consists of the reaction of a lipid with an alcohol, preferably of low molecular weight, to produce an ester and a by-product, glycerol. The global process is developed through three reversible and consecutive reactions in which the intermediate products formed are diglycerides and monoglycerides (Figure 3).

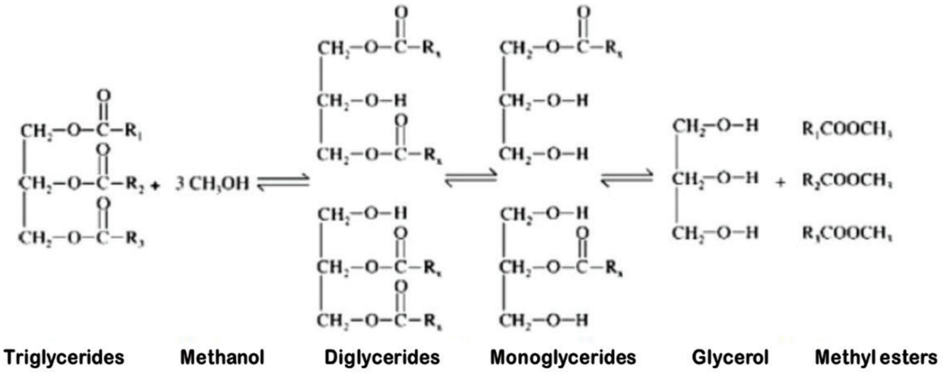

Figure 3. General transesterification reaction. Modified from [19].

Stoichiometrically 3 moles of alcohol are required for each mole of triglyceride, but in practice an excess of alcohol should be used to increase the formation of esters and facilitate the separation of glycerin. Higher reaction temperatures speed up the reaction and shorten the reaction time [20,22].

The use of ethanol is preferable in the transesterification reaction, because it can be generated from agricultural products, it is renewable and environmentally friendly, however, methanol is the alcohol most used for its physical, chemical and low-cost advantages [20].

The dry biomass was removed from the Petri dish using a spatula and placed in thin cloth bags, to carry out the extraction of lipids using the Soxhlet equipment and hexane as a solvent, in accordance with the requirements of the Mexican standard [21] as an analysis method for the determination of recoverable fats and oils in natural, waste and treated wastewater. It should be noted that the treated water is analyzed at the UAM-Azcapotzalco wastewater treatment plant and in our case the donated water was only sterilized in an autoclave to later use it as a culture medium.

In this work, the method of chemical transesterification with methanol was used employing sodium hydroxide as catalyst [22]. Sodium hydroxide was placed in a grill and a magnetic stirrer was placed, then methanol was added.

Once the oil and sodium methoxide have the same temperature (60 °C), at which the reaction was carried out, the methoxide was added to the oil obtained from *Botryococcus braunii* and allowed to react for 10 minutes.

2.7. *Determination of amount of catalyst (sodium hydroxide)*

A basic catalyst (NaOH Sigma-Aldrich) was chosen as they have been reported to be more effective than acid catalysts and enzymes; the recommended amount of catalyst is between 0.1 and 1% w / w of oils and fats [22]. Thus, the amount of NaOH was determined as follows:

Amount of NaOH = Weight of oil (g) * (0.1-1% w / w of oil and fats).

2.8. *Determination of amount of methanol*

As mentioned before stoichiometrically 3 moles of alcohol are required for each mole of triglyceride, however using an excess of alcohol allows to increase the formation of esters and facilitate the separation of glycerin, so in this stage the ratio of methanol 6: 1 was used [22,23].

Once the biodiesel was obtained, it was placed in a settling funnel for 24 h to separate from the glycerin that was obtained as a by-product of the reaction [22,23].

The biodiesel obtained was characterized by means of Infrared PerkinElmer FT-IR Spectrometer Frontier equipment, which allowed to verify that it contains the functional groups of biodiesels. The characteristic signs of biodiesel are two groups of bands of own absorption of the methyl esters that make it up, in the region of the fingerprints the band appears between (1200-1300) cm-1 originated by the asymmetric axial deformation C-O and in the region of the functional groups between 1750 cm-1 and 1730 cm-1 is the intense peak corresponding to the group carbonyl (C = O) typical of esters; which is related to the vibration of relatively constant and interference-free stretching, this signal being the greater difference with the spectrum of diesel; for both spectra the absorption band between 2950 and 3000 cm-1, corresponding to the stretching of the CH3, CH2 and CH bonds of aliphatic carbons [5,22].

Gas chromatographic analysis of biodiesel was performed on a PerkinElmer Clarus 580 gas chromatograph, by a temperature ramp of 10°C/min, from 160°C to 230°C for 8 min, with an injector temperature of 225°C, 1mL / min Helium flow and an Elite-Wax column of 30 m long and a maximum temperature of 240°C [5].

2.9. *The Bio-Jet fuel obtention*

The biodiesel obtained from renewable sources can be used in aviation engines either as the only component or in mixtures with conventional kerosene; the main aim is to obtain biofuels from products that do not compete with the fertile land necessary for food production; however, to achieve truly sustainable production, methodologies must be urgently implemented of third generation biofuels such as microalgae.

In this work, the biodiesel obtained from the algae was distilled using a vacuum pump and micro-distillation equipment. The boiling range was 60-70ºC. Once the distillation was carried out, a light fraction and another heavy fraction were obtained. The light fraction is bio-jet fuel.

The Biodiesel yield was obtained according to Cao *et al.,* 2013 [12].

The bio-jet fuel sample was characterized using the Perkin Elmer Frontier brand FT-IR Frontier Spectrometer as follows, 20 µL of sample was used, and a scan was made in the wavelength range of 400 cm-1 to 4000 cm-1.

3. Results and discussion

3.1. *Physicochemical parameters in photobioreactors*

The temperature oscillated from 20-25 ° C; The pH presented stable dynamics at the beginning of the process, rising from 8 to 10 in the first ninety days of growth, however after 60 days of growth the pH varied in each one in ranges from 7 to 11, possibly due to the solubility, ionization state and bioavailability of nutrients in the medium, during the growth time of *B. braunii* [15].

3.2. Botryococcus braunii *biomass*

The growth of the biomass in the photobioreactor of 5%V MgO NPs reached a maximum concentration of 60 mg/ml, during the first 80 days of growth, as well as the growth kinetics of the control treatment and the treatment with 20 % MgO NPs presented similar kinetics during the first eighty days, growing dramatically towards the last forty-five final days, reaching a concentration of 130 mg/ml for the control treatment, at 125 days and for the treatment of 20% MgO NPs concentrations were obtained up to 80 mg/ml, for the treatment of 10% *B. braunii* a similar growth was obtained in the last stage reaching concentrations of 67 mg/ml at 119 days, the photobioreactor with 10% MgO NPs was the one with the lowest concentration of biomass and the photobioreactor without MgO NPs was the one with the highest (Figure 4).

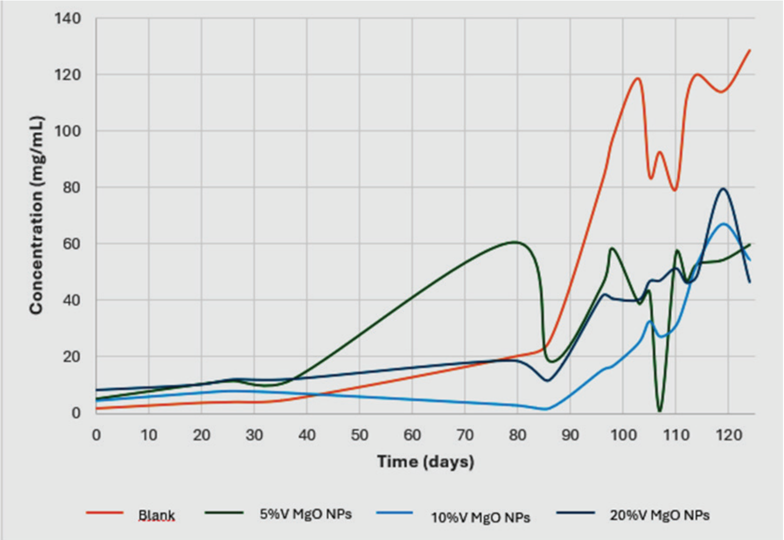

Figure 4. Growth kinetics in the different treatments with NPs of MgO.

The highest concentration of lipids was obtained in the 10% treatment with MgONPs, reaching a total of 3.1 g, and for the photobioreactors without NPs a maximum concentration of 2.77 g lipids was present (Fig. 5).

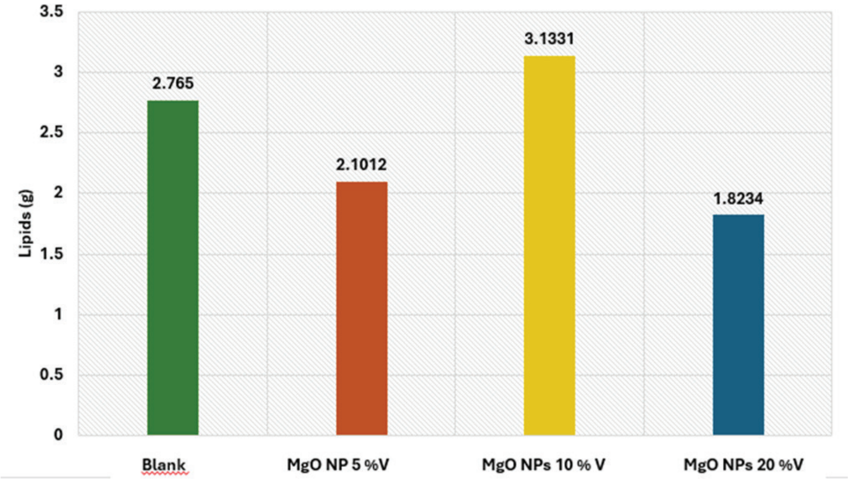

Figure 5. Lipids obtained from the culture of *B. braunii* at different concentrations of MgO nanoparticles.

3.3. *Analysis of variance of growth kinetics*

The analysis of variance showed significant differences between the growth concentration of *B. braunii* of the control treatment with respect to the other treatments with MgO NPs ($p = 0.000002$), with respect to the concentrations between 5 %V MgO NPs, and 20 %V MgO NPs presented significant differences, observing the 5 %V with higher concentrations with respect to those of 10 %V and 20 %V (Figure 6).

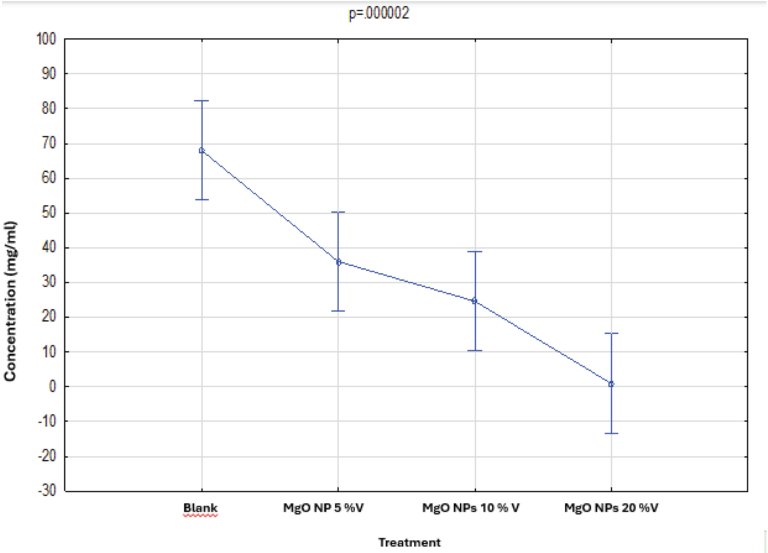

Figure 6. Analysis of variance of the growth kinetics between the different concentrations of NP´s MgO.

These results indicate that, even though more biomass is obtained, more lipids that can be used in transesterification are not necessarily obtained. On the other hand, the percentage of MgO NPs is of great relevance, since as we have seen there is a concentration where more biomass is obtained (blank and 5 %V), but there is a concentration where more lipids are obtained (10 %V), and this is evident since the differences between treatments are statistically significant [5,22].

Furthermore, MgO NPs are influencing both the growth and lipid production of *B. braunii* even perhaps in an easier obtaining of lipids for transesterification by the granzyme-perforin-dependent mechanism, in accordance what is reported by Al-Omar *et al.*, 2021[24] for 9-13 nm flake-type graphene oxide nanoparticles.

3.4. *MgO Nanoparticles (MgO NPs) characterization*

The synthesized MgO nanoparticles were characterized by UV-Vis spectroscopy, the maximum wavelength was 209.49 nm (Figure 7).

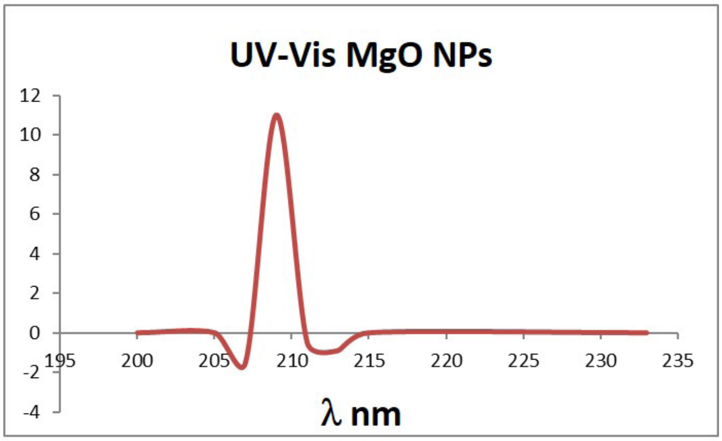

Figure 7. Uv-Vis spectroscopy of MgO Nanoparticles.

This result indicates that sodium citrate acts as a reducing and stabilizing agent [9].

The characterization by TEM shows that we obtained “flake-like” particles of approximately 10 nm in diameter (Figure 8 A-C).

Al-Omar *et al.*, 2021[24] report graphene oxide flake nanoparticles of 9-13 nm, that increased the activation of phagocytic cells by augmenting the cell mortality through the granzyme-perforin-dependent mechanism. This is a promising strategy for controlling cancer cells. Perforins are proteins that integrate into the cell membrane and induce the formation of pores that cause osmotic lysis of the

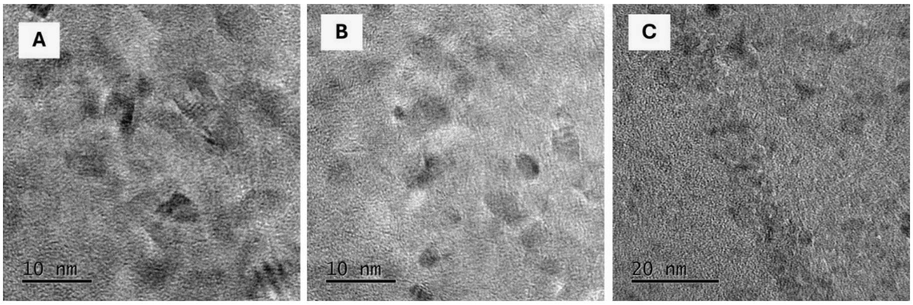

Figure 8. Shows the "flake-like" shape of approximately 10 nm magnesium oxide nanoparticles A-B. In panel C a view with the 20 nm measurement.

target cell. Meanwhile, granzymes enter the cell through endocytosis and induce apoptosis. In our case, these mechanisms could facilitate the exit of lipids from the algae for the subsequent transesterification process.

3.5. *Biodiesel*

The biodiesel obtained by transesterification was characterized using infrared spectrometry equipment (Figure 9). The characteristic signals of biodiesel are two groups of absorption bands of the methyl esters that make it up. In the fingerprint region, the band appears between (1200-1300) cm-1, caused by the asymmetric C-O axial deformation, and in the region of the functional groups between 1750 cm-1 and 1730 cm-1 there is the intense peak corresponding to the carbonyl group (C=O) typical of esters; which is related to the relatively constant and interference-free stretching vibration, this signal being the biggest difference with the diesel spectrum; For both spectra, the absorption band between (2950

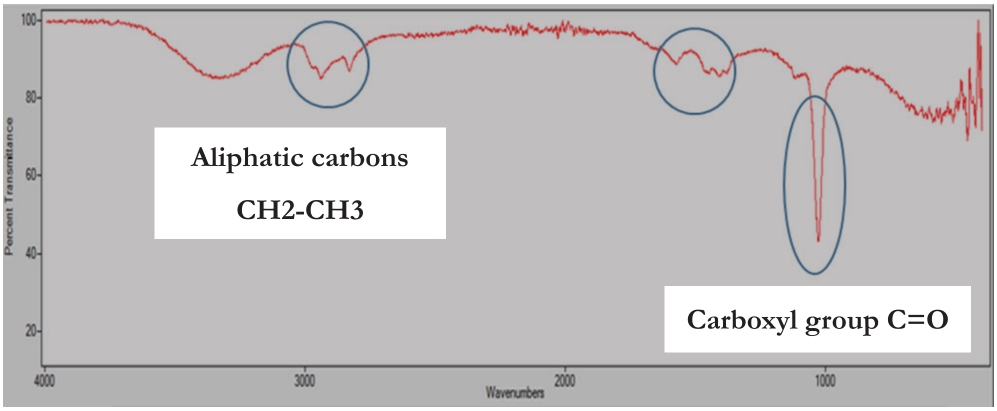

Figure 9. IR spectrum of the biodiesel obtained.

and 3000) cm-1 is common, corresponding to the stretching of the CH3, CH2 and CH bonds of aliphatic carbons, like Chávez-Sandoval *et al.,* 2021 a [5].

As we know, biodiesel absorbs in the region of 2,750 cm-1 -3,000 cm-1 corresponding to the vibration modes of -CH2 and -CH3. As the triglycerides present in the oil are converted to methyl esters when the reagent is methanol, the -CH3 groups increase in biodiesel composed mainly of fatty acid alkyl esters and therefore the intensity of the peak by ~ 2924 cm-1 also increases [5,25].

3.5.1. Biodiesel yield

The biodiesel yield from microalgae biomass was calculated with the following formula:

$$\text{Biodiesel yield }(\%) = \frac{\textit{Biodiesel mass g}}{\textit{algae mass }(g) * \textit{ oil content }(\%)} \;*100$$

In this work we obtained a biodiesel yield were 86%. Has been reported that these values over 100% are obtained when processing the well-dried biomass, since when the humidity increases the yield drops considerably, and because perhaps other molecules such as phospholipids, or accumulation of lipids on the cell surface, are also transformed into biodiesel [5, 12, 26].

3.6. *Bio-jet fuel*

Figure 10 shows the result of the characterization of bio-jet fuel obtained, using IR. With respect to the IR of biodiesel, a change is noted in the intensity of the 1200 cm-1 band and in the bands from 2950 to 3000 cm-1, which indicates the consumption of the hydroxyl group and the formation of hydrocarbons, at 1730 cm -1 only two bands are observed and there was an increase in the intensity of the 1750 cm-1 band, indicating the conversion of biodiesel into bio-jet fuel.

Bio-jet fuel obtained from the microalgae *B. braunii* is a good option to mitigate the problems surrounding fossil fuels and with this help contribute to the production of new fuels derived from third generation biomass [27]. Likewise, this work shows a path to innovate techniques that allow greater efficiency in the biokerosene production process, furthermore, if a direct transesterification were carried out, costs would also be reduced in the production of these biofuels, as reported by Chavez-Sandoval *et al.*, 2021a and Wang *et al.,* 2022 [5,28]. Insomuch as the American Society for Testing and Materials (ASTM) has certified several biofuel production technologies including kerosene, since hydroprocessed esters and fatty acids can now be commercialized [5, 26, 28].

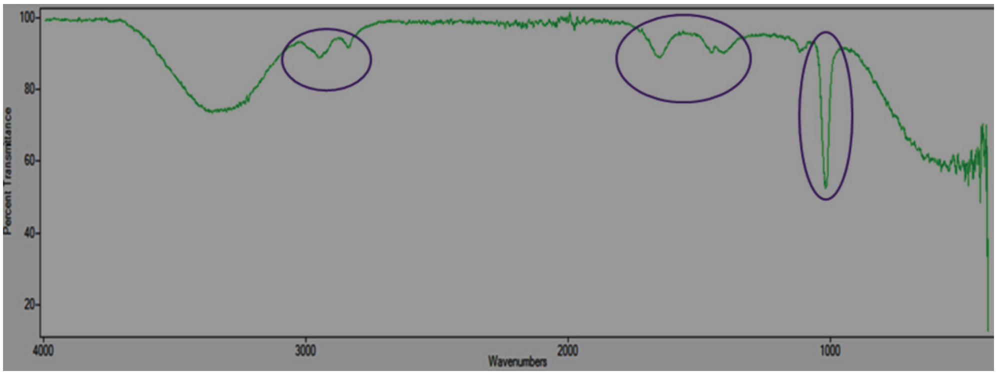

Figure 10. IR spectrum of bio-jet fuel obtained.

4. Conclusions

Botryococcus braunii UTEX 2441 was cultivated in domestic wastewater, thus reducing costs.

MgO NPs were obtained with a maximum wavelength of 209.5 nm. sodium citrate acts as a reducing and stabilizing agent.

TEM characterization showed that the obtained magnesium oxide nanoparticles (MgO NPs) are approximately 10 nm in size and flake shaped.

The effect of the MgO NPs depends on the concentration, since in the photobioreactor without MgO NPs (Blank) greater biomass growth was observed, but in the photobioreactor with 10% MgO NPs a greater amount of lipids was obtained.

We obtained a biodiesel yield were 86%. Since when the humidity increases the yield drops considerably. However perhaps other molecules such as phospholipids, or accumulation of lipids on the cell surface, are also could transformed into biodiesel. Furthermore, if a direct transesterification were carried out, costs would also be reduced in the production of these biofuels.

Bio-jet fuel obtained from the microalgae *B. braunii* is a good option to mitigate the problems surrounding fossil fuels and with this help contribute to the production of new fuels derived from third generation biomass.

This work shows a path to innovate techniques that allow greater efficiency in the biokerosene production process; Considering the economic characteristics of developing countries and even the maturity of technology; bio-kerosene production is a promising short-term alternative.

All authors have read and agree to the manuscript and there is no conflict of interest between them, any additional information or data may be requested from the corresponding author (JABL).

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